Reversing Cholinergic Bronchoconstriction by Common Inotropic Agents: A Randomized Experimental Trial on Isolated Perfused Rat Lungs

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BACKGROUND: The ability of inotropic agents to alter airway reactivity and lung tissue mechanics has not been compared in a well-controlled experimental model. Therefore, we compared the potential to alter lung tissue viscoelasticity and bronchodilator effects of commonly used inotropic agents in an isolated perfused rat lung model.

METHODS: After achieving steady state lung perfusion, sustained bronchoconstriction was induced by acetylcholine (ACh). Isolated rat lungs were then randomly allocated to 6 groups treated with either saline vehicle (n = 8) or incremental concentrations of inotropes (adrenaline, n = 8; dopamine, n = 7; dobutamine, n = 7; milrinone, n = 8; or levosimendan, n = 6) added to the whole-blood perfusate. Airway resistance (Raw), lung tissue damping (G), and elastance were measured under baseline conditions, during steady-state ACh-induced constriction and for each inotrope dose.

RESULTS: No change in Raw was observed after addition of the saline vehicle. Raw was significantly lower after addition of dopamine (maximum difference [95% CI] of 29 [12–46]% relative to the saline control, $P = .004$), levosimendan (58 [39–77]%, $P < .001$), and adrenaline (37 [21–53]%, $P < .001$), whereas no significant differences were observed at any dose of milrinone (5 [−12 to 22]%) and dobutamine (4 [−13 to 21]%). Lung tissue damping (G) was lower in animals receiving the highest doses of adrenaline (difference: 22 [7–37]%, $P = .015$), dobutamine (20 [5–35]%, $P = .024$), milrinone (20 [6–34]%, $P = .026$), and levosimendan (36 [19–53]%, $P < .001$) than in controls.

CONCLUSIONS: Although dobutamine and milrinone did not reduce cholinergic bronchoconstriction, they reversed the ACh-induced elevations in lung tissue resistance. In contrast, adrenaline, dopamine, and levosimendan exhibited both potent bronchodilatory action against ACh and diminished lung tissue damping. Further work is needed to determine whether these effects are clinically relevant in humans. (Anesth Analg XXX;XXX:00–00)

KEY POINTS

- **Question:** Does the potential to alter lung tissue viscoelasticity and bronchodilator properties of routinely administered inotropic agents differ?
- **Findings:** Adrenaline, dopamine, and levosimendan exhibited potent bronchodilatory action, whereas dobutamine and milrinone did not. All tested agents but dopamine reduced lung tissue damping.
- **Meaning:** Potentially relevant effects of common inotropic agents on lung mechanics may have clinical relevance in patients who require both cardiovascular and respiratory support.

Inotropic agents are commonly used to treat hemodynamically unstable patients. However, such cardiovascular support drugs may also affect lung function indirectly via effects of cardiopulmonary interactions on lung tissue[1] and directly via effects on bronchial smooth muscle.[2,3] Although dopamine act mainly on dopaminergic receptors,[2,4,5] dobutamine stimulates primarily the β_1 receptor subtype,[6] adrenaline binds to α and β receptors, milrinone inhibits the phosphodiesterase 3 (PDE3) pathway,[7] and levosimendan acts via ATP-dependent potassium channels that repolarize the bronchial smooth muscle cell.[8] Despite the potential of these receptor-based effects to affect lung mechanics, their effects on lung function have not been systematically compared.

In vivo studies of the effects of inotropes on lung mechanics are confounded by several factors. These include marked alterations in systemic and pulmonary hemodynamics,[9] modulation of pulmonary nervous control,[10] and effects on lung water clearance.[10,11] We, therefore, chose to use isolated perfused rat lungs because this experimental setup allows the pulmonary system to be examined while excluding the effects of different systemic or neurological factors. We hypothesized that inotropes differ in their

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ability to relax airway smooth muscle and alter lung parenchymal viscoelasticity.

METHODS

Ethics

The experimental protocol was approved by the Experimental Ethics Committee of the University of Geneva and the Animal Welfare Committee of the Canton of Geneva, Switzerland (no GE/2/16, January 26, 2016). All procedures were performed according to the current animal protection laws of Switzerland (LPA, RS455) and reported in compliance with ARRIVE guidelines.

Animal Preparations

Adult male Sprague-Dawley rats (n = 44, weighing 516 ± 101 g) were anaesthetized by inhaled isoflurane through a facemask (3%–4% induction dose). Tracheostomy was performed with a 14-gauge polyethylene cannula (B. Braun Melsungen AG, Melsungen, Germany). Mechanical ventilation was initiated with a tidal volume of 7 mL/kg, a positive end-expiratory pressure of 2.5 cm H_2O, an inspired oxygen fraction of 0.5, and a respiratory rate of 70–80/min by means of a constant volume cycled rodent ventilator (model 683, Harvard Apparatus, South Natick, MA). Anesthesia was maintained with 1.4% inhaled isoflurane. The femoral artery and vein were prepared surgically (28-gauge catheters, Portex, Hythe, United Kingdom) for blood sampling and continuous blood pressure monitoring using a calibrated pressure transducer (Honeywell, model 156 PC 06-GW2, Zürich, Switzerland). After full anticoagulation (1.5 IU/g intravenous [IV] heparin), 20–25 mL blood was gently withdrawn via the arterial catheter over 5 minutes, while continuously replacing the collected blood with a constant rate IV infusion of hydroxyethyl starch (130/0.4 6%, Voluven 6%, Fresenius Kabi, Bad Homburg, Germany) to maintain a constant intravascular volume and a systemic blood pressure above 50 mm Hg to minimize ischemic lesions of the lung during this normovolemic hemodilution procedure. This procedure resulted in the collection of approximately 20 mL blood with a hematocrit of approximately 30%–35%, which was used to prime the perfusion.

Preparation of Isolated Lungs

Lungs were harvested as described previously.[12,13] Briefly, after the administration of IV fentanyl (15 µg/kg), a median sternotomy was performed, and the main pulmonary artery was cannulated through the right ventricular outflow track (14-gauge, B. Braun Melsungen AG). The left ventricle was also catheterized via a left ventriculotomy, in which a Combifix Adapter (B. Braun Melsungen AG) was tightly fixed and connected to medical-grade silicone tubing. A third catheter was placed directly in the left atrium (cat no 602235; internal diameter, 1.47 mm; external diameter, 1.96 mm; Ulrich, St. Gallen, Switzerland). The lungs and the heart were excised and extracted as a single block, dissected free of adjacent tissue.

Lung Perfusion

The lung perfusion setup used in this study has been described in detail previously.[13] The excised heart–lung block was placed in a humidified, preheated (37°C) Plexiglas chamber (590 mL). Volume-controlled positive-pressure mechanical ventilation was applied by a rodent ventilator (Inspira ASV, Harvard Apparatus, South Natick, MA) at a respiratory rate of 30/min, a tidal volume of 7 mL/kg, and a positive end-expiratory pressure of 5 cm H_2O. Ventilation was performed with a gas mixture containing 50% O_2 and 5% CO_2 to avoid hypocapnia. After the initiation of mechanical ventilation, 2 hyperinflation maneuvers (double tidal volume) were applied to eliminate the atelectatic areas. Lung perfusion was initiated by setting the pulmonary arterial pressure (Ppa) to 25 mm Hg hydrostatic pressure and left atrial pressure (Pla) to 8 mm Hg by placing arterial and venous reservoir tanks at appropriate heights. Both Pla and Ppa were greater than the mean airway pressure (Paw), and the lungs were therefore maintained under West's zone 3 conditions (Ppa > Pla > mean [Paw]). The blood exiting the left ventricular outflow cannula was collected in a cylinder and aspirated from this reservoir with polyethylene tubing passing through a roller pump (Ismatec Pump, Glattbugg, Zurich, Switzerland). The Ppa and Pla values were recorded (Honeywell, model 156-PC 06-GW2), and pulmonary blood flow (Qp) was measured continuously with a transit time flowmeter (T-201 CDS, Transonic Systems Inc, Ithaca, NY) situated between the perfusion reservoir and the catheter cannulating the main pulmonary artery. The pulmonary vascular resistance (PVR) was calculated by determining the pressure difference between Ppa and Pla and dividing this value by Qp. Pulmonary capillary pressure (Pc) was estimated by applying the Gaar equation ($Pc = Pla + 0.44 \times [Ppa - Pla]$).[14] Paw, Ppa, Pla, Pc, Qp, PVR, and lung weight, obtained by using an isometric force displacement transducer (Grass FT03, Quincy, MA), were monitored and recorded continuously on a microcomputer at a sampling rate of 100 Hz via an analog/digital interface converter (Powerlab 16/35, ADInstruments, Oxfordshire, United Kingdom).

Lung Mechanical Measurements

The input impedance of the lung (ZL) measured by forced oscillations[15] was used to estimate pulmonary mechanical parameters. Briefly, the measurements were performed during end-expiratory apneic periods of 8 seconds. A loudspeaker and the tracheal cannula were connected with a polyethylene wave tube (length, 102 cm; internal diameter, 0.2 cm), and a small amplitude (1 cm H_2O peak to peak) forcing signal was delivered into the trachea. The pregenerated pseudorandom forcing signal included 23 sinusoidal components ranging from 0.5 to 20.75 Hz. Lateral pressures were measured at both ends of the wave tube with miniature side arm transducers (model 33NA002D, ICSensors, Milpitas, CA). These pressure signals were low-pass filtered at 25 Hz and digitized at a sampling frequency of 128 Hz. The pressure transfer function was created by fast Fourier transformation, and ZL was computed as the load impedance of the wave tube by using the transmission line theory.[16]

A well-validated mathematical model[15] containing airway and constant-phase tissue compartments was fitted to the averaged ZL data in each condition by minimizing the squared sum of weighted differences between the measured and the modeled values. The airways were characterized by

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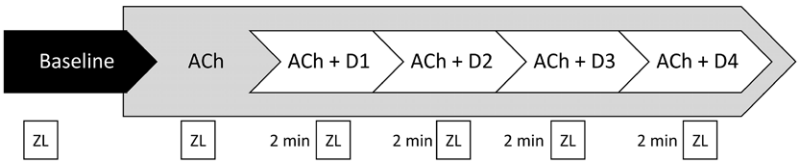

Figure 1. Scheme of the experimental protocol. ACh indicates acetylcholine alone; ACh+D1–D4, increasing doses of saline or inotropic agents (dopamine, dobutamine, adrenaline, milrinone, and levosimendan, respectively) during sustained ACh infusions; ZL, a set of lung impedance recordings.

frequency-independent airway resistance (Raw) and airway inertance (Iaw), whereas the tissue compartment was described by the parenchymal damping (G) and elastance (H).[17] The lung tissue hysteresivity (η) was calculated as G/H.[18] The airway parameters were corrected for the resistance and inertance of the tracheal tube.

Study Protocol

The timeline of the study protocol is illustrated in Figure 1. After the establishment of steady-state hemodynamic parameters, 2 recruitment maneuvers were performed: application of a double tidal volume to the standardize volume history and full recruitment the lungs. Baseline lung mechanical parameters were measured by recording a set of ZL data epochs including 4–6 recordings. A continuous infusion of acetylcholine (ACh) was initiated via the pulmonary arterial line to achieve a sustained elevation of bronchial tone. The rate of ACh infusion was adjusted until Raw was approximately doubled, resulting in infusion rates of 3–7 µg/h. When lung mechanical parameters were reproducible at this elevated level, another set of ZL data was recorded to characterize the response to ACh itself. Lungs were then randomly included in 1 of the 6 protocol groups for addition of a drug to the 20-mL perfusate: dopamine (15, 30, 60, and 120 µg, equivalent to 9×10^{-8} to 8×10^{-7} M, n = 7), dobutamine (20, 40, 80, and 160 µg, equivalent to 7×10^{-8} to 5×10^{-7} M, n = 7), adrenaline (0.2, 0.4, 1.0, and 10 µg, equivalent to 10^{-9} to 5×10^{-8} M, n = 8), milrinone (10, 20, 40, and 80 µg, equivalent to 10^{-9} to 5×10^{-8} M, n = 8), levosimendan (50, 100, 500, and 1250 µg, equivalent to 2×10^{-7} to 5×10^{-6} M, n = 6), and a control group treated with the saline vehicle at equivalent injected volumes (40, 80, 160, and 320 µL, n = 8). The selection of the doses for each drug was based on the relevant concentrations that demonstrated hemodynamic effects in a similar experimental setting.[19,20] A set of ZL data was recorded at each dose after a 2-minute stabilization period.

Statistical Analyses

Differences between parameter values are expressed as mean difference and 95% CI. The Shapiro-Wilk test was used to test data for normality. Two-way repeated measures analysis of variances (ANOVA) were applied to compare the mechanical or hemodynamic parameters and to assess group-by-time interactions with variables of group allocation as independent and the applied doses as within-group factors. Pair-wise comparisons were performed by using the Holm-Sidak method.

The study was designed to have sufficient power (80%) to be able to detect a difference equal to 25% of the mean of one of the groups in the percentage change of the primary outcome parameter Raw. Accordingly, sample size estimation indicated that 6 animals were required in each group to detect a significant difference between the protocol groups by using ANOVA tests. This estimation is based on our previous data obtained in similar experimental conditions,[21] with a mean expected difference of 25%, an assumed standard deviation of change in Raw of 11% after ACh challenges in isolated perfused rat lungs, a power of 80%, and a significance level of 5%.

The statistical tests were performed with the SigmaPlot statistical software package (version 13, Systat Software, Inc Chicago, IL), with a significance level of $P < .05$ in each test.

RESULTS

Figure 2 summarizes changes in lung mechanical parameters when ACh was administered alone and when normal saline or the different inotropes were added to the perfusate. ACh infusion induced significant and stable changes in the airway and lung tissue mechanics in the saline-treated control group. Statistically significant interactions between the ACh dose as within-group factor and the treatment as between-group factor were observed for Raw ($P < .001$) and Iaw ($P = .003$). Airway mechanical parameters (Raw and Iaw) did not differ between the saline-treated control group and groups treated with milrinone and dobutamine. However, when compared to the saline-treated control groups, Raw was lower after adrenaline (maximum between-group difference in the absolute values of 20 [6–34] $cmH_2O{\cdot}s/L$, $P = .025$), dopamine (17 [3–31] $cmH_2O{\cdot}s/L$, $P = .048$), and levosimendan treatments (29 [14–44] $cmH_2O{\cdot}s/L$, $P = .002$). The absolute values of the mechanical parameters representing tissue mechanics (G, H, and η) did not differ between groups.

To clarify the pulmonary effects of the inotropic drugs in the presence of elevated bronchial tone, the changes in lung mechanical parameters were also expressed relative to those values obtained when only ACh was infused into the perfusate (Figure 3). Saline administration did not affect airway and lung tissue parameters with the exception of H, where small increases were observed at the end of the protocol. The interaction between the ACh dose as within-group factor and the treatment as between-group factor was statistically significant for the relative changes in Raw ($P < .001$), Iaw ($P < .001$), G ($P = .019$), and H ($P = .048$). Compared to the saline-treated control group, significantly smaller changes in Raw were observed after adrenaline (maximum between-group difference in the relative changes of 37 [21–53]%, $P < .001$), dopamine (29 [12–46]%, $P = .004$), and levosimendan (58 [39–77]%, $P < .001$) treatments. However, neither milrinone (5 [−12 to 22]%) nor dobutamine (4 [−13 to 21]%) had any effect on Raw at any dose. Dopamine treatments elevated Iaw compared to the saline-treated control group (maximum between-group difference of 0.034 [0.014–0.054] $cmH_2O{\cdot}s^2/L$, $P = .025$). The ACh-induced changes in G were lower than those in the control group of lungs at the highest doses of adrenaline (between-group difference of 22 [7–37]%, $P = .015$), dobutamine (20 [5–35]%, $P = .024$),

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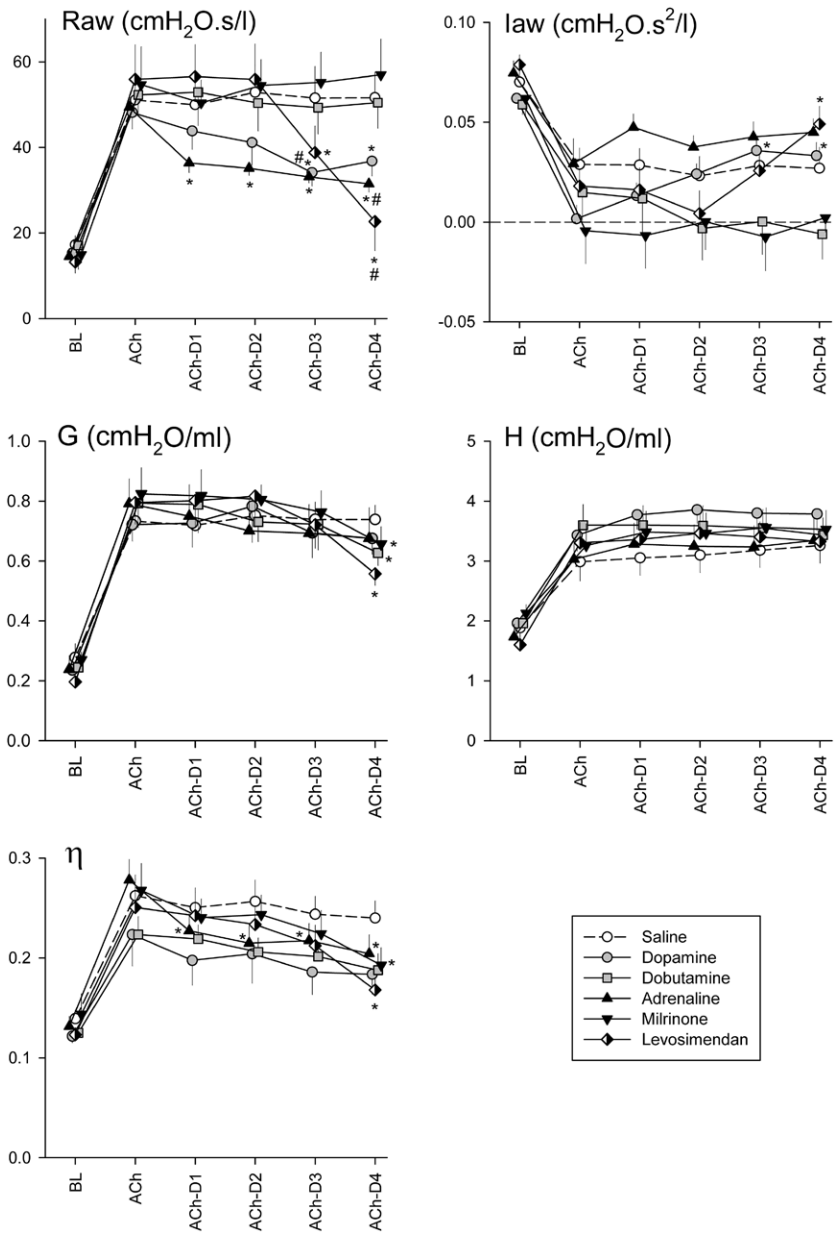

Figure 2. Changes in the lung mechanical parameters. ACh indicates acetylcholine alone; ACh+D1–D4, increasing doses of saline or inotropic agents during sustained ACh infusions; BL, baseline; G, lung tissue damping; H, lung tissue elastance; Iaw, airway inertance; Raw, airway resistance; η, lung tissue hysteresivity. *P < .05 versus ACh within a group (BL not indicated); #P < .05 versus saline group within a condition.

milrinone (20 [6–34]%, P = .026), and levosimendan (36 [19–53]%, P < .001). Smaller changes in H were only obtained for dobutamine compared to saline treatments (17 [6–28]%, P = .017), whereas η was significantly reduced after milrinone (18 [5–31]%, P = .026), levosimendan (23 [8–38]%, P = .018), and adrenaline (20 [7–33]%, P = .022) treatments.

Pulmonary hemodynamic variables at different time points in the protocol are depicted in Figure 4. Pc did not differ statistically between the groups and exhibited no significant changes throughout the study. There was a significant interaction between the ACh dose and the treatment for all measured pulmonary hemodynamic variables (P < .001 for all) demonstrating that inotropes affected the magnitude of ACh-induced changes. PVR increased in lungs treated with adrenaline and dopamine (P < .001), resulting in higher values than in saline controls with differences between groups of 1.8 (0.7–2.9; P = .011) and 2.7 (1.5–3.9) mm Hg.min/mL (P < .001), respectively. Pulmonary blood flow (Qp) was lower in the adrenaline group than the saline group (between-group difference at the highest dose of 6.8 [2.6–11.0] mL/min, P = .015), whereas Qp was higher after administration of levosimendan (5.2 [1.1–9.3] mL/min, P = .049).

The relationship of changes in resistive parameters of the vascular (PVR) and bronchial (Raw) compartments is displayed in Figure 5. In the case of dopamine and adrenaline, bronchodilation was associated with marked pulmonary vasoconstriction. However, levosimendan induced marked bronchodilation with no concomitant pulmonary vascular changes.

DISCUSSION

In this experimental rat model, we characterized the effect of common inotropic agents on lung mechanics. We found

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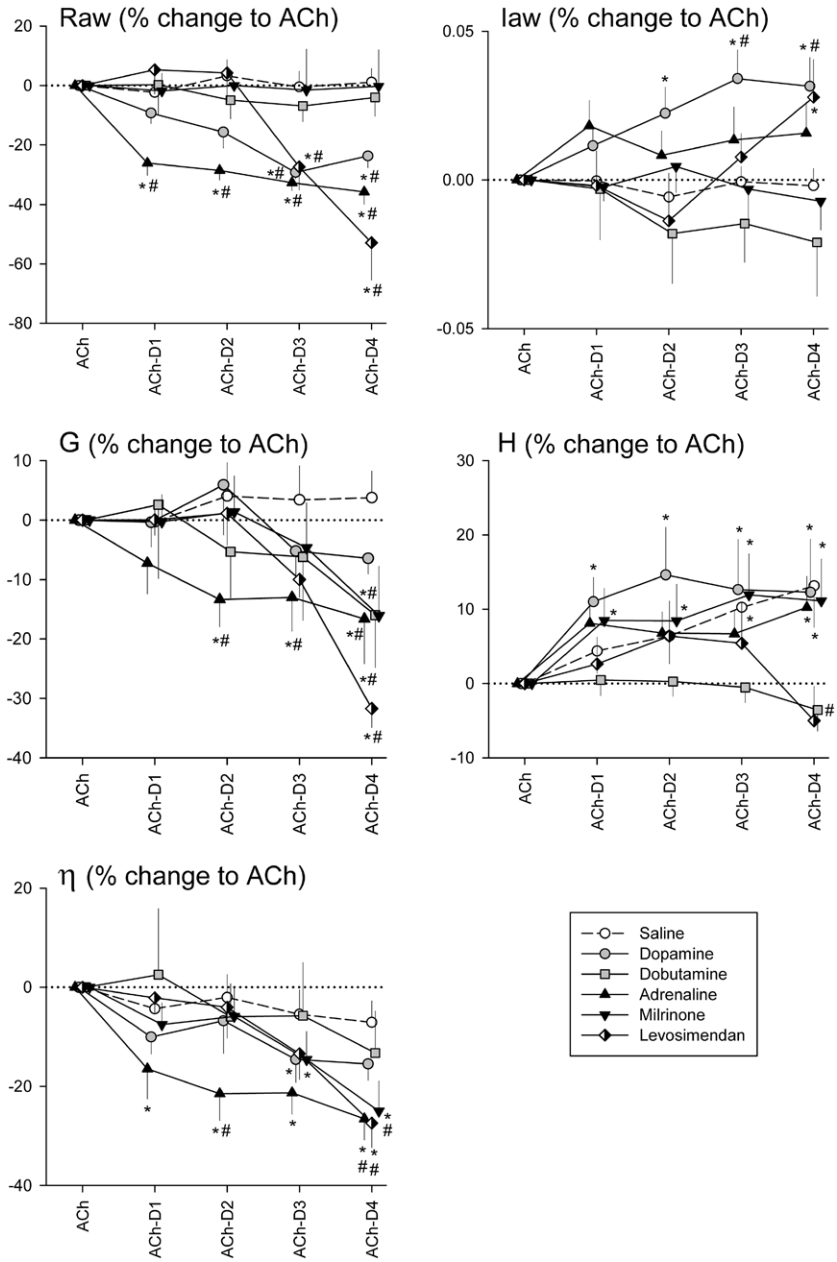

Figure 3. Comparison of changes in lung mechanical parameters exposed to ACh only or ACH and an inotropic agent. ACh indicates acetylcholine alone; ACh+D1–D4, increasing doses of inotropic agents during sustained ACh infusions; G, lung tissue damping; H, lung tissue elastance; Iaw, airway inertance; Raw, airway resistance; η: lung tissue hysteresivity. *$P < .05$ versus ACh within a group; #$P < .05$ versus saline group within a condition.

that inotropes differ considerably in their relaxing effects on the increased Raw caused by ACh administration. Although dobutamine and milrinone were unable to reverse the elevated airway tone, adrenaline, dopamine, and levosimendan exhibited significant bronchodilatory potentials. These lung mechanical changes were associated with marked pulmonary vasoconstriction only for adrenaline and dopamine.

Since the isolated perfused rat lung model controls for cardiopulmonary interactions, it has been used previously to establish the direct effects of bronchoactive agents on the lung.[21] Our forced oscillatory parameters agree with earlier data with respect to baseline conditions,[12,21] but differ somewhat with respect to changes in tissue stiffness with ACh provocation.[21] This dissimilarity may possibly be due to bolus injection of ACh used in earlier studies, whereas continuous infusion was applied in the present protocol. The latter induces a longer, steady-state effect with more severe sustained deteriorations of the lung tissue mechanics.

Although adrenaline, dopamine, and levosimendan affect smooth muscle via fundamentally different mechanisms; all reversed ACh-induced bronchoconstriction. However, effects of these 3 inotropes on lung mechanical parameters differed. Unlike adrenaline, dopamine and levosimendan reversed ACh-induced decreases in Iaw and increases in G (Figure 2). Although Iaw in the single-compartment model is related to the mass of the gas in the airways and G reflects lung tissue damping, decreases in Iaw associated with elevations in G are hallmarks of heterogeneities in peripheral ventilation.[15,22] Cholinergic challenge in our study likely induced peripheral airway constriction, causing similar ventilation heterogeneities. In this context, adrenaline caused predominantly central bronchodilation

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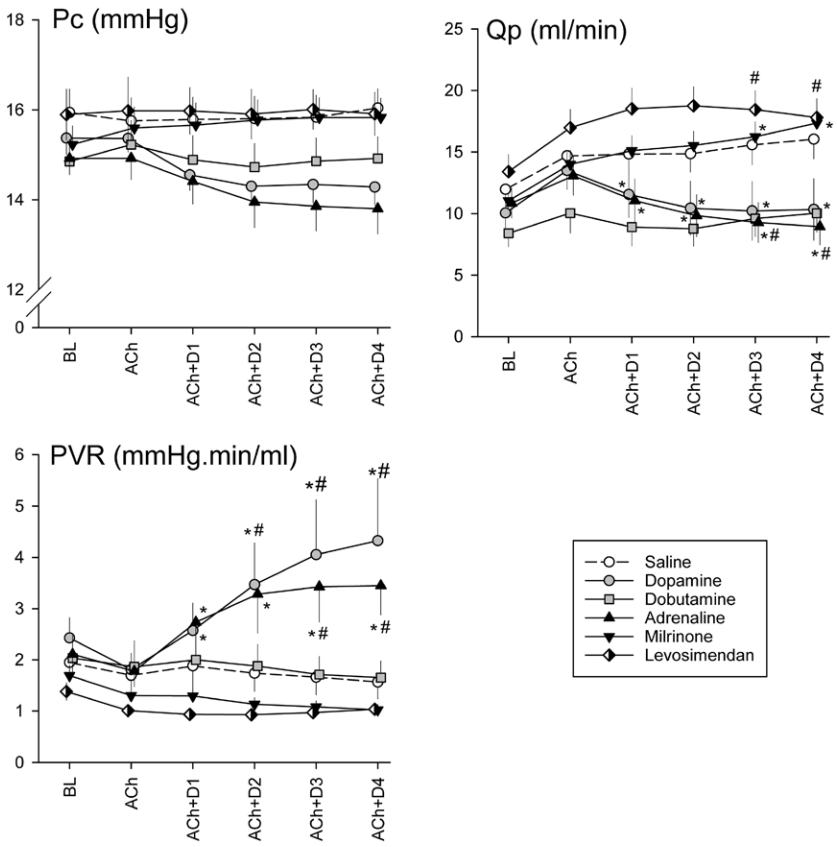

Figure 4. Pulmonary hemodynamic parameters. ACh indicates acetylcholine alone; ACh+D1–D4, increasing doses of inotropic agents during sustained ACh infusions; BL, baseline; Pc, pulmonary capillary pressure; PVR, pulmonary vascular resistance; Qp, pulmonary blood flow. *P < .05 versus ACh within a group (BL not indicated); #P < .05 versus saline group within a condition.

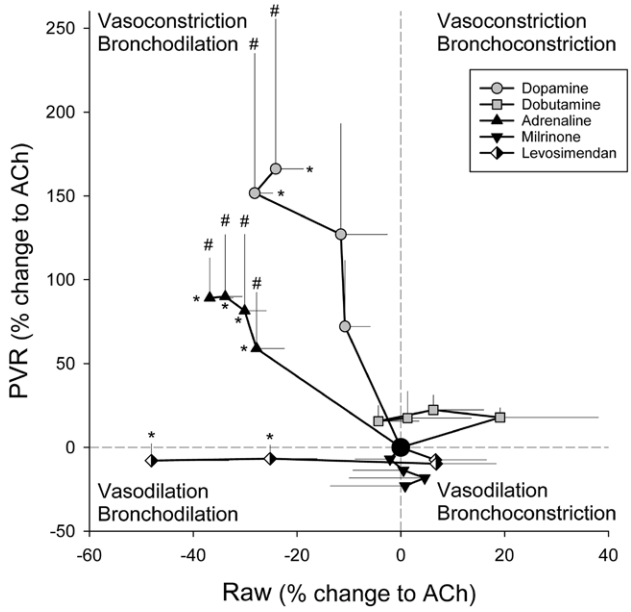

Figure 5. Relationship of the resistive changes in the pulmonary vasculature and the bronchi relative to their values obtained with ACh only. ACh indicates acetylcholine; PVR, pulmonary vascular resistance; Raw, airway resistance. *P < .05 in Raw. #P < .05 in PVR.

as demonstrated by the marked decreases in Raw and the lack of change in Iaw and G. Conversely, for levosimendan and dopamine, the central airway dilation was associated with reduced peripheral ventilation heterogeneities, as evidenced by the reversal of ACh-induced changes in Iaw and G. This observed effect of adrenaline is in accordance with previous results demonstrating the limited ability of β_2 stimulation to resolve lung peripheral constriction.[23]

We included adrenaline in this study as a reference drug, as its bronchodilator effects have been well established in earlier studies.[3,24] Our findings are consistent with the expected effect, which supports the suitability of our experimental model. The pulmonary effects of dopamine are controversial, with some studies reporting dopamine-induced bronchoconstriction[25] and others demonstrating either a lack of airway effect[26] or bronchodilation.[5] Our results suggest that dopamine has bronchodilatory effects, as it reversed cholinergic constriction of both central and peripheral airways in our study. The difference in airway effects of dopamine and adrenaline suggests that dopamine-induced bronchodilation may be mediated by dopaminergic receptors,[2,27] whereas dilatory beta adrenergic effects of dopamine[28] play a smaller role. Our observation that levosimendan reversed cholinergic bronchoconstriction is also consistent with a 2009 guinea pig study[8] and a 2017 in vivo rabbit trial.[29] Interestingly, unlike dopamine and adrenaline, which exert dose-dependent bronchial effects, levosimendan appears to have a threshold concentration above which its bronchodilator ability becomes apparent (Figure 3).

Our findings demonstrate the lack of any bronchodilatory potential for dobutamine at relevant doses. This finding supports the minor role of β_1 receptors in the modulation of airway tone and also indicates that the β_2-mediated effect of dobutamine[30] was not detected as a change in lung mechanical properties. Similarly, milrinone, a PDE3 inhibitor, had

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a neutral effect on airway mechanics but improved lung tissue damping. This effect may be related to the relaxation of contractile elements in the lung parenchyma during induced constriction and/or a decrease of the intrinsic energy loss inside the lung tissues.[31] Furthermore, the decreased ventilation heterogeneities in the lung periphery[22] and the mild pulmonary vasodilation with subsequent increase in Qp (Figure 4) may have also contributed to this effect. This finding should be considered in the context of the relaxant effects of PDE3 inhibitors, which appeared only at much higher and supraclinical concentrations (>10^{-7} M) than those applied in the present study,[32] and depend on the species and the bronchoconstrictive agents applied.[33]

In addition to the lung mechanical properties, the present experimental setting allowed the assessment of the direct pulmonary hemodynamic responses to inotropes. In agreement with previous investigations, adrenaline and dopamine exhibited potent pulmonary vasoconstrictor responses.[34,35] Since ACh was administered in all preparations, we did not aim to address the vasodilation activity of the inotropes. Despite this restriction, we did note a slight pulmonary vasodilation with administering milrinone, levosimendan, and dobutamine as Qp increased with all 3 inotropes. These findings are in agreement with the pulmonary vasodilatory effects established earlier with these agents.[36,37] Interestingly, we found remarkable differences in the responses of the vascular and bronchial compartments of the lung to the inotropes we tested (Figure 5). The potent bronchodilatory abilities of adrenaline and dopamine were associated with marked pulmonary vasoconstriction, thereby demonstrating opposite effects on the 2 compartments. Conversely, the strong bronchodilatory effect of levosimendan appeared to be in concordance with its vasodilator potential, suggesting similar responses for the vascular and bronchial smooth muscles.

An important limitation of our study is that although the concentrations we used correspond to earlier studies using ex-vivo models[19,20] and in rat models in vivo,[38–40] comparison to human doses is not straightforward. The concentrations of milrinone and adrenaline used in this study approximate the medium-range clinical doses and are at the high end of the clinically applied dosage for dopamine, dobutamine, and levosimendan. The use of different doses of the various inotropes limits the comparability of their effects on the pulmonary mechanics and circulation. Therefore, differences in the effects of the different inotropes we observed should be considered cautiously, and these limitations prevent ready extrapolation of our findings to a clinical setting.

In summary, our data demonstrate that adrenaline, dopamine, and levosimendan exert a bronchodilatory effect in the presence of cholinergic elevation of the airway smooth muscle tone. Conversely, dobutamine and milrinone had no bronchodilatory properties. Although adrenaline, dopamine, and levosimendan exert direct airway smooth muscle effects via different pathways, they all had bronchodilatory effects in the presence of sustained cholinergic bronchoconstriction. These agents had different effects on the vascular smooth muscle; however, levosimendan exhibited a vasodilatory effect in contrast to adrenaline and dopamine, which induced vasoconstriction. The multifactorial effect of these commonly used inotropic agents may have particular relevance in patients with concomitant impairments in the circulatory and respiratory systems, for which cardiovascular support and improved respiratory function have mutual importance. ■

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